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Traction, Trypsin, and Tensegrity

Iva Marija Tolić–Nørrelykke[1] and Ning Wang[2]

[1] Rugjer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia
[2] Harvard School of Public Health, 665 Huntington Ave., Boston, MA 02115, USA

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Traction, Trypsin, and Tensegrity[#]

Iva Marija Tolić–Nørrelykke[1,*] and Ning Wang[2]

[1] Rugjer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia
[2] Harvard School of Public Health, 665 Huntington Ave., Boston, MA 02115, USA

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Abstract

Motivation. Cells exert traction on their substrates. These mechanical stresses are crucial in cell contraction, locomotion, growth, and differentiation.

Method. The cells are plated on a flexible gel substrate with embedded fluorescent microbeads. The traction of the cells induces a deformation of the gel and hence displacement of the microbeads. These displacements are used to compute the traction by Fourier Transform Traction Cytometry (FTTC).

Results. The FTTC method was tested using computer simulations. The method was applied to cultured human airway smooth muscle cells before and after their partial detachment from the substrate, induced by trypsin. Before detachment, the traction was highest at the cell ends, whereas there was no traction after partial detachment.

Conclusions. Adherent cells are tensed and attached to the substrate mainly at their ends. After one end of the cell detaches, the cell rebounds like a spring to its intact attachment site. The cells typically retract towards the site of highest traction, possibly because this is the site of strong attachments. Retraction of the cell after partial detachment is consistent with the tensegrity model of cell mechanics, in which tension in the cytoskeleton is crucial for the mechanical stability of cells.

Keywords. Traction microscopy; mechanical stress; cytoskeleton; cell contraction; adhesion; retraction.

Abbreviations and notations

HASM, human airway smooth muscle	FTTC, Fourier Transform Traction Cytometry

1 INTRODUCTION

Living cells adherent to a surface exert mechanical forces upon the surface. These forces are important in many cell functions, including cell crawling, growth, programmed cell death (apoptosis) and gene expression [1,2]. Several experimental approaches have been developed to measure cell traction [3–5]. In the polyacrylamide gel method [4,6], the cells adhere to the surface of a flexible gel. Cellular traction induces deformation of the gel. Fluorescent microbeads

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* Correspondence author; current address: LENS European Laboratory for Non–Linear Spectroscopy – Polo Scientifico, Via Nello Carrara 1, 50019 Sesto Fiorentino (FI), Italy; phone: 39–055–457–2482; fax: 39–055–457–2451; E–mail: tolic@lens.unifi.it.

embedded below the surface of the gel serve as markers of the gel deformation.

We have developed an exact computational method to calculate the traction from the displacement field of the gel, based on recasting the relationship between displacement and traction in Fourier space [7]. This method, named Fourier Transform Traction Cytometry (FTTC), was used to measure traction in single human airway smooth muscle (HASM) cells [8–12].

In this paper we (*i*) test the FTTC method by performing computer simulations, and (*ii*) measure the traction exerted by adherent cells before and after their partial detachment from the substrate. We find that, after one end of the cell detaches, the cell rebounds like a spring to its intact attachment site. Retraction of the cell after partial detachment is consistent with the tensegrity model of cell mechanics. According to this model, the tension in the actin cytoskeleton is crucial for the mechanical stability of cells. This tension is balanced by traction on the substrate, and, to a smaller extent, by intracellular structures that resist compression [10].

2 MATERIALS AND METHODS

2.1 Computational Methods

2.1.1 Determination of the traction field

The first step in determination of the traction field is computation of the displacement field. The displacement field between each image of the microbeads and the reference image (*i.e.*, the image recorded after the cell detached completely from the gel) was determined using the Image Correlation Method [7,11]. In short, the images were divided into small window areas. The displacement field between a pair of images was obtained by identifying the coordinates of the peak of the cross–correlation function between each pair of window areas.

Next, the traction field was calculated from the displacement field using Fourier Transform Traction Cytometry [7]. This calculation is based on recasting the Boussinesq solution for the displacement field on a surface of a semi–infinite solid, given the surface traction [13], into Fourier space [7]. The spatial resolution of the traction maps was 2.7 μm.

2.1.2 Computer simulations

The projected area of an imaginary cell was designed as a 40 μm × 20 μm rectangle. An artificial traction map was constructed within the projected area of the cell: a lattice with a spacing of 2.7 μm was defined and the traction was represented as point–forces in the centers of the lattice divisions. The lattice spacing was chosen to be the same as in the experiments (2.7 μm = 16 pixels). X–traction was chosen to be a linear function of the *x*–coordinate, whereas *y*–traction was set to zero. The largest x–force was comparable to the force measured in cells, 2.86 nN, which corresponds to a

traction of 400 Pa. Next, the displacements were calculated on the lattice using the Boussinesq solution [13]. Poisson's ratio was 0.48, and the Young's modulus 1300 Pa.

To test the effect of the cell boundary on the traction recovered using Constrained FTTC, false cell boundaries were constructed. In one case the cell boundary was drawn too large, and in the other case too small (10 μm error on each side of the cell). The traction was then recovered from the displacements using Constrained FTTC. All calculations were performed in MATLAB (MathWorks).

2.2 Experimental Methods

2.2.1 Polyacrylamide gel substrates

The polyacrylamide gel discs with embedded fluorescent microspheres were prepared as described previously [4,6,9]. In short, a mixture of acrylamide (2%), bis–acrylamide (0.25%), and fluorescent polystyrene microbeads (0.2 μm in diameter) was put on a glass coverslips. The droplet of the solution was covered by a small circular coverslip and the assembly was turned upside down. After polymerization, the circular coverslip was removed and type–I collagen, 0.2 mg/ml, was attached to the surface of the gel. Young's modulus of the gel was determined to be 1300 Pa. Poisson's ratio of the gel was taken to be 0.48.

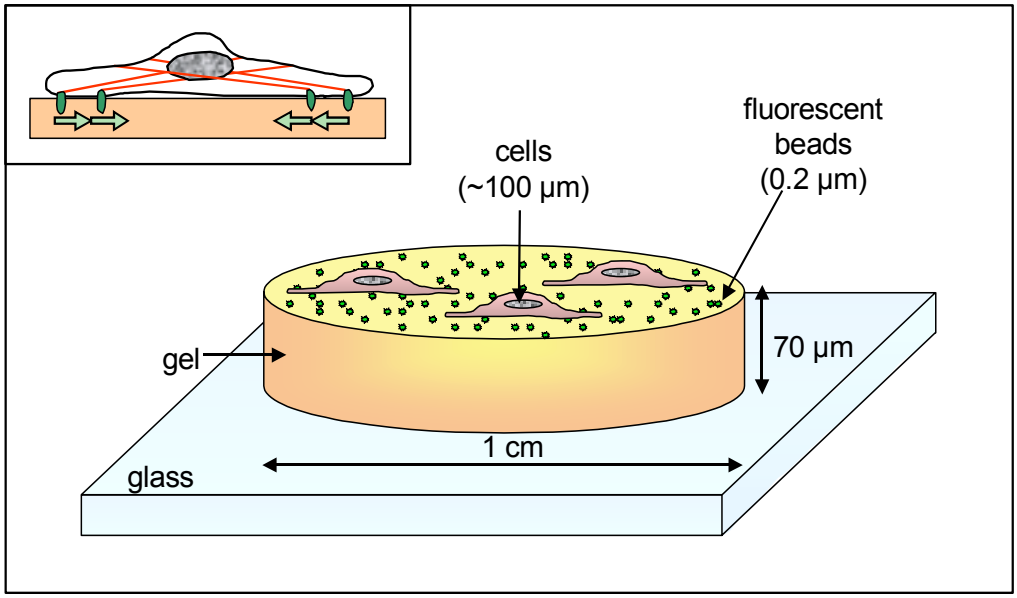

Figure 1. Experimental setup. The cells are plated on a flexible gel disc with embedded fluorescent microbeads. Cellular traction forces induce deformation of the gel and hence displacement of the microbeads. These displacements are used to compute the traction. Inset: a cell exerting traction (green arrows) on the gel. The force is generated by actin filaments (red lines) and transmitted to the gel via integrin receptors (green ovals).

2.2.2 Cell culture and microscopy

The experimental protocol was as described earlier [9,11]. HASM cells were serum deprived for 2 days before the experiments. The cells were plated on a gel disc in a serum–free medium and allowed to spread for 6 hours (Figure 1). A plastic dish with a gel disc in the medium was mounted on the microscope stage. The cells were stimulated with graded doses of histamine (0.1, 1, and 10 μM) at 2 minutes intervals, after which they were left for about 10 minutes. At last, trypsin was added to detach the cells from the substrate. The experiment was terminated when the cell in the field of view dissociated from the gel, thus leaving the gel with no surface traction. Phase contrast images of the cells and fluorescence images of the microbeads were taken interchangeably every 20 seconds.

3 RESULTS AND DISCUSSION

3.1 Results of Computer Simulations of Traction

Fourier Transform Traction Cytometry (FTTC) is an exact solution to the problem of determination of the traction field exerted by an adherent cell, given the displacement field of the flexible substrate. The method is divided into two cases, Unconstrained and Constrained FTTC [7]. The difference between these two cases is the following. In Unconstrained FTTC, the traction is computed directly from the displacement field in one step, without imposing constraints on the resulting traction field. Simple and straight–forward as it is, this method may generate traction on the gel surface outside the region of the cell–gel contact. This is an undesirable feature insofar as the cell is the sole source of traction on the surface of the gel. In other words, we assume that the traction outside the cell boundary is zero.

Constrained FTTC was developed as a method that imposes the constraint of zero–traction outside the cell boundary on the resulting traction field. The computational procedure of Constrained FTTC consists of the determination of the cell boundary using a phase–contrast image of the cell, and the iterative calculation of the traction, where the traction outside the cell boundary is zero and the displacements within the cell boundary match the measured displacements in that region [7].

Although Constrained FTTC is often the preferred method for the calculation of traction, the resulting traction field may contain artifacts associated with the cell boundary. Namely, ambiguities often arise in determination of the cell boundary from an image of the cell. Well spread cells typically have flat lamellipodia and thin protrusions whose tips are not clearly visible in phase contrast images. We therefore performed computer simulations in order to assess the effect of an incorrectly determined cell boundary on the resulting traction field.

A simulated traction field is shown in Figure 2A. Colors represent the magnitude of traction. The color scale to the right gives the mapping between the colors and the traction values. The arrows show the direction and relative magnitude of traction. For visual clarity, the arrows have been thinned by a factor of 2. The white line marks the cell boundary.

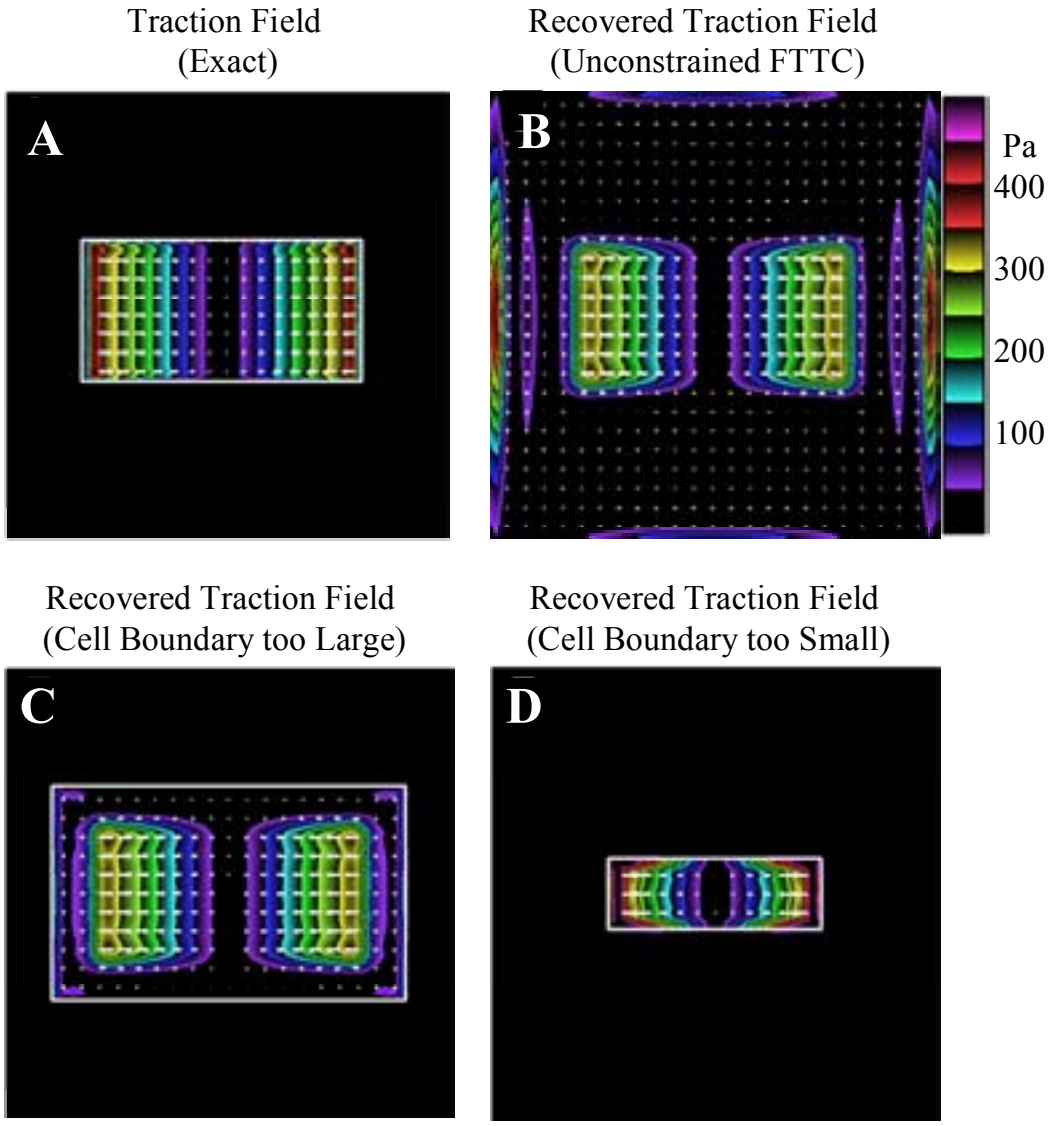

Figure 2. Computer simulations of cellular traction. (A) An artificial traction map. (B) Traction recovered from the displacements induced by the traction shown in panel A, using Unconstrained FTTC. (C) Traction recovered from the same displacements as for panel B. Here, Constrained FTTC was used. The cell boundary was drawn too large. (D) Same as in panel C, but the cell boundary was drawn too small. Colors show the absolute magnitude of traction (see color bar); arrows show the direction and relative magnitude of traction. The white rectangles mark the cell boundary. Note that in Constrained FTTC with an enlarged cell boundary, as well as in Unconstrained FTTC, the recovered traction resembles well the original traction field. On the contrary, shrinking the cell boundary in the constrained case induced high traction at the new cell boundary.

Next, the displacements induced by the traction shown in Figure 2A were computed and used as a simulation of an experimentally measured displacement field. The traction was calculated from those displacements using Unconstrained FTTC (Figure 2B). As stated above, this method does not require information about the location of the cell boundary. The recovered traction field (Figure 2B) is not significantly different from the original one (Figure 2A), except for the traction at the boundary of the field of view, which stems from the periodic boundary conditions in Fourier space [7], and does not affect the traction of the cell.

Subsequently, an erroneous cell boundary was defined by enlarging the original cell boundary (compare the white rectangle in Figures 2A and 2C). Figure 2C shows the traction recovered from the simulated displacement data using Constrained FTTC. Note that drawing the cell boundary too big did not introduce significant errors in the traction field, *i.e.*, the traction fields in Figures 2A and 2C are similar. Figure 2D shows the traction recovered in a similar way as in Figure 2C, but using a cell boundary that is much smaller than the original one (compare the white rectangles in Figures 2A and 2D). Drawing the boundary too small introduced serious errors in the traction field: traction at the new cell boundary was artifactually high.

We conclude that, if in doubt regarding the exact location of the cell boundary, it is better to define the cell boundary somewhat larger. All parts of the cell that may exert force on the substrate are then found within the boundary. Otherwise, the resulting traction field contains false high traction at the false cell boundary, as a compensation for real traction at locations that have been excluded from the erroneously small cell projected area.

3.2 Experimentally Measured Cell Traction

3.2.1 Traction vanishes after partial detachment of the cell

HASM cells exert traction on the substrate during histamine–induced contraction [9,11]. The traction is usually highest close to the cell ends, and directed towards the cell center. Figure 3A shows an image of a cell adherent to a flexible gel disc. The cell was treated by histamine and is contracting, as inferred from the traction pattern shown in Figure 3B. Other cells showed similar traction fields. Each experiment was terminated by treating the cell with trypsin in order to detach the cell from the gel and to free the gel from surface traction. The cells did not, however, detach immediately after the addition of trypsin. Before complete detachment of the cell from the gel, in 30% of the cells partial detachment was observed. The cell detached at one side and retracted towards the other side, where the attachment was still intact. The cell was then approximately round. Its surface was covered by membrane evaginations. A few minutes later the cell detached completely from the gel surface and disappeared from the field of view.

Figure 3C shows the cell from Figure 3A after partial detachment from the surface induced by trypsin. After trypsin treatment, the cell was much shorter than before treatment, and attached at

only one of the old cell ends (the upper right end). Figure 3D shows the corresponding traction field. The traction in Figures 3B and 3D was calculated using Unconstrained FTTC. Constrained FTTC yielded similar results (not shown). Whereas large traction was visible under the cell before treatment with trypsin (Figure 3B), there was no traction associated with the projected area of the cell after the treatment (Figure 3D). The traction was zero across the whole area of Figure 3D, except for a very small traction in some regions, which were not correlated with the cell location. That traction was due to measurement noise.

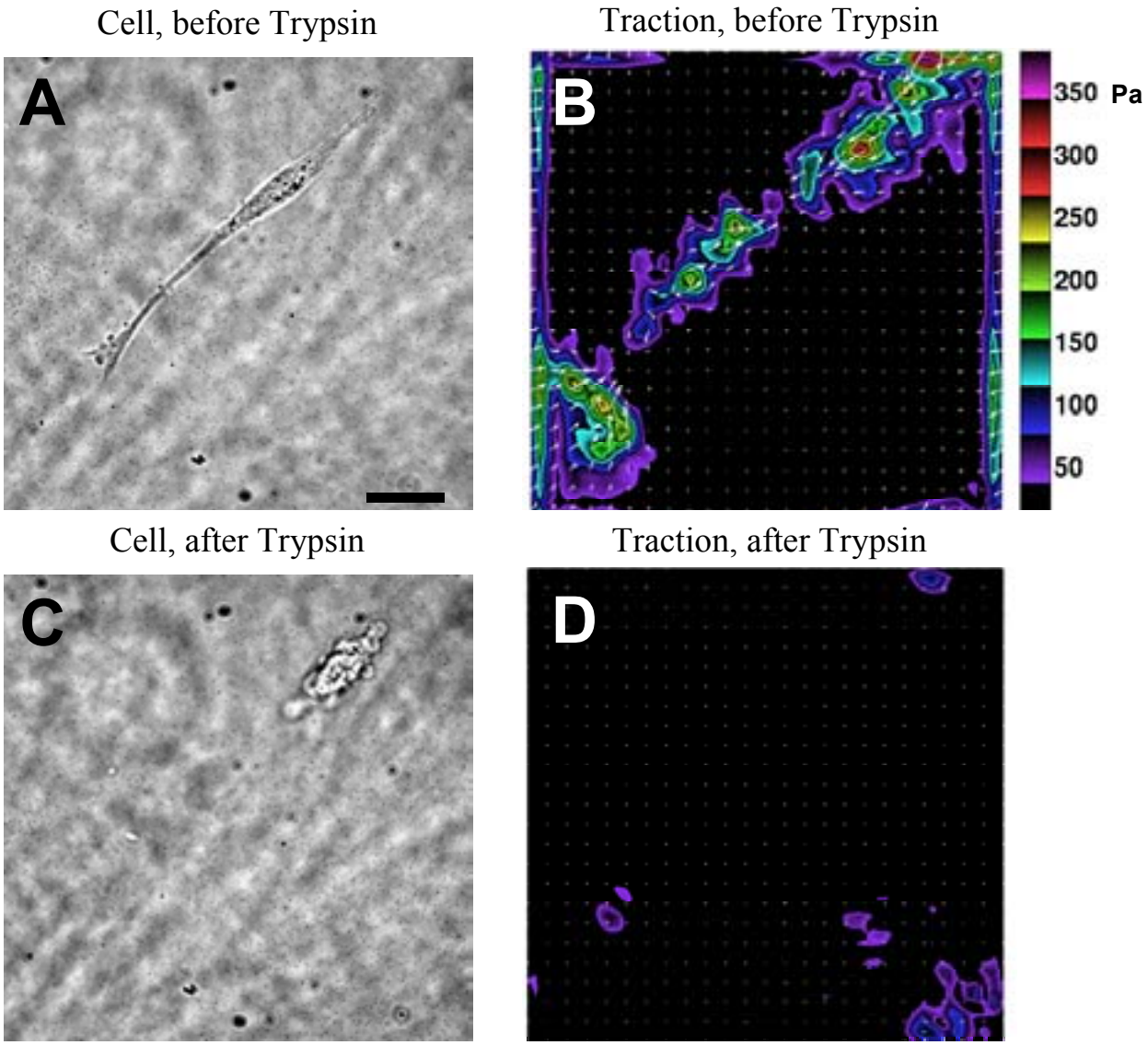

Figure 3. **Traction of a cell vanishes after treatment with trypsin**. (A) Phase contrast image of the cell before the addition of trypsin, and (B) the corresponding traction field. Colors show the absolute magnitude of traction (see color bar); arrows show the direction and relative magnitude of traction. (C) The same cell as in panel A, 3 minutes after the addition of trypsin, and (D) the corresponding traction field. The traction fields were obtained using Unconstrained FTTC. Scale bar in A, 20 μm.

The sudden retraction of a cell may be attributed to the release in tension within the cytoskeleton after a part of the cell detaches from its adhesion sites on the gel. The cell then quickly retracts as would a tensed rubber band. Similar retraction of adherent cells was observed when endothelial cells were cut or detached from their basal surface by a microneedle [14]. The retraction was prevented when the cells were pretreated with cytochalasin D to disrupt actin filaments. These results suggested that there is tension in adherent cells, and that the tension is primarily supported by the actin lattice [14].

The zero traction fields measured after treatment with trypsin (Figure 3D) are consistent with the tensegrity model of cell mechanics [8,15]. Nevertheless, other models that depict the cell as a tensed structure, *e.g.* a fluid–filled balloon, cannot be ruled out based on this result alone. Observations consistent with the tensegrity model, but not with continuum balloon–type models, have been described in Ref. [8].

According to the tensegrity model, the cell maintains its shape stability through tension in the actin cytoskeleton. The tension is balanced by intracellular structures that resist compression, *e.g.* microtubules [10], and by traction at the interface between the cell and the substrate. In the specific tensegrity model applicable to the cells studied here, the adherent cell is tensed and attached to the surface primarily at its two ends. After one end detaches, the cell rebounds like a spring to its intact attachment site (Figure 4). Since the cell is then attached at one side only, it exerts no surface traction upon the gel any more.

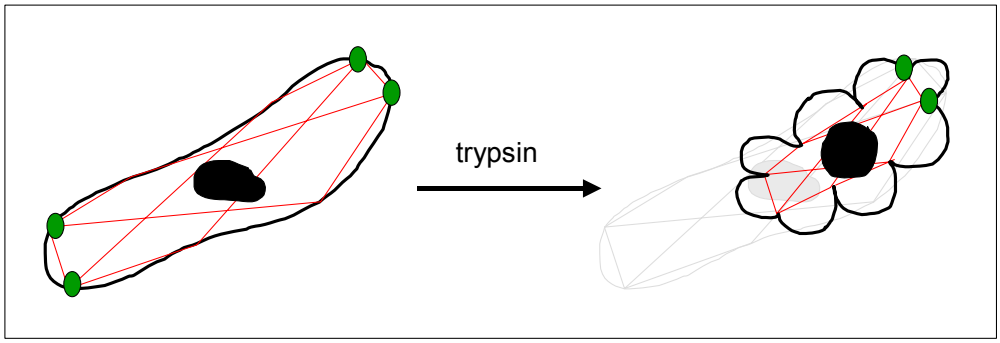

Figure 4. **A model of a contracted cell**. Before treatment with trypsin (drawing on the left), the cell is tensed and spread on the gel surface. The main attachments to the gel are at the cell ends (green ovals). The tension is produced by the actin filaments (red lines). Treatment with trypsin induces loss of contact between the cell and the substrate at one end of the cell, hence the cell retracts towards the other end (drawing on the right). Membrane evaginations appear between neighboring attachment sites of the contractile units (red lines) to the cell membrane (black line), to accommodate the extra volume of the shortened cell. Cell nucleus (black area) and the original cell position (gray lines in the drawing on the right) are also shown.

3.2.2 Membrane evaginations accommodate the displaced cell volume

After treatment with trypsin, the cells assumed a nearly round shape with numerous membrane evaginations (blebs) covering its surface (Figure 3C). Formation of membrane evaginations has been reported before [16, 17]. For example, evaginations of portions of the cell membrane were observed in isolated smooth muscle cells after shortening in response to brief electrical stimulation [16]. The evaginations were seen during the contraction, and disappeared again during subsequent relaxation and elongation of the cell. The formation of evaginations is thought to be the consequence of the cell contraction. Contractile units are attached to the cell membrane at discrete sites. As the cell contracts, these units pull on the cell membrane. Only the portions of the cell membrane that do not contain the attachment sites for the contractile units are free to move outwards in order to accommodate the volume displaced by the shortening of the cell [16], see Figure 4.

3.2.3 Regions with highest traction detach last

Is the probability of detachment correlated with traction? In 8 out of 10 cells the protrusion with the highest traction force before trypsin treatment was the one that detached last. This was the location towards which the cell retracted. The cell from Figure 3 is an example of such behavior. In the 2 remaining cells a small region away from the cell ends, which exerted significant but not highest traction, was the site towards the whole cell retracted.

The tip of the protrusion with the highest traction might be the site where thick actin bundles end in strong focal adhesions. Stronger attachments between the cell and the gel with multiple integrin–collagen bonds may be more resistant to the proteolytic activity of trypsin.

Detachment of the cell from its substrate is a crucial step during cell migration. To migrate, cells must make stable contacts with the substrate, exert traction, contract, and partly detach from the substrate. In migrating cell, strong traction is exerted near the leading edge of the cell [18]. The tail region, which shows low traction [18,19], is the site of repeated detachment between the cytoskeleton, adhesion receptors, and the cell substrate [20]. Thus detachment during both migration and trypsinization occurs in the regions of low traction.

4 CONCLUSIONS

We measured traction in adherent cells before and after their partial detachment from the substrate, induced by treatment with trypsin. The zero traction measured after the partial detachment is consistent with the tensegrity model, in which the adherent cell is tensed. After one end of the cell detaches, the cell rebounds like a spring to its intact attachment site, and the gel substrate assumes its original shape free of surface traction. The cells typically retract towards the

site of highest traction. Finally, this work confirms that Fourier Transform Traction Cytometry is a useful and reliable method for computing cell traction.

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Biographies

Iva Marija Tolić–Nørrelykke is a research assistant at the Rugjer Bošković Institute in Zagreb, Croatia. She obtained a Ph.D. degree in biology–biomathematics in 2002 from the University of Zagreb, under the supervision of Prof. Nenad Trinajstić and Prof. Ning Wang. Dr. Tolić–Nørrelykke is currently working with Prof. Francesco Pavone at LENS, Firenze, Italy, on mechanics of cell division and cell motility.

Ning Wang is an associate professor at Harvard University School of Public Health. His research interests are cell mechanics and mechanotransduction. Prof. Wang obtained his doctor of science degree in physiology at Harvard in 1990 and became a faculty member at Harvard in 1994.